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# THE EFFECT OF NEWBOULDIA LAEVIS ON WISTAR RAT'S TESTIS AND LIVER HISTOMORPHOLOGY IN CADMIUM-INDUCED TESTICULAR TOXICITY AND HEPATOTOXICITY

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## ABSTRACT

**Background:** *Newbouldia laevis* is a plant known for its great nutritional and medicinal importance with antimicrobial, antibacterial, anti-inflammatory, anti-malarial, anti-coagulant, anti-diabetic and anti-oxidant properties. Cadmium is globally acknowledged as an environmental intoxicant with widespread toxicity on the biological system. Thus this work was carried out to evaluate the protective role of *Newbouldia laevis* aqueous leaf extract on the wistar rat testis and liver histomorphology in cadmium-induced testicular toxicity and hepatotoxicity. **Methodology:** This experimental study was carried out on 25 male adult wistar rats. The animals were divided into 5 groups of 5 animals each – Control, Cadmium, *Newbouldia laevis*, Co-administration and Pre/Post Treatment Groups. The Treatment Groups received 1.2mg/kg body weight of cadmium, 200mg/kg body weight of *Newbouldia laevis*, 1.2mg/kg body weight of cadmium + 200mg/kg body weight of *Newbouldia laevis* simultaneously and 1.2mg/kg body weight of cadmium for two weeks and 200mg/kg body weight of *Newbouldia laevis* for two weeks. **Results:** The exposure of experimental animals to cadmium showed deleterious effects on the organs of study (testes and liver). Treatment with aqueous extract of *Newbouldia laevis* revealed varying degrees of restoration after

hepatotoxicity by cadmium. However, *Newbouldia laevis* was unable to ameliorate the testicular damage occasioned by cadmium as cellular disruptions were still evident even with its administration. **Conclusion**: The present study suggests that the oral administration of aqueous leaf extracts of *Newbouldia laevis* can be used to manage hepatotoxicity but not testicular toxicity.

**KEYWORDS:** Cadmium, *Newbouldia laevis*, Histomorphology, Testes, Liver, Hepatotoxicy, Testicular Toxicity.

## INTRODUCTION

*Newbouldia laevis* (NBL) plant belongs to the magnoliophyta phylum and the bignoniaceae family with a wide array of medicinal applications. Its bark is analgesic, stomachic. A decoction is used in the treatment of coughs, diarrhoea and dysentery, whilst it is also given to children for epilepsy and convulsion. A decoction of the bark combined with chillies, is used in the treatment of chest pains (Akerele *et al.*, 2011). The bark is used to cure a range of skin conditions including septic wounds, abscesses and ulcers; and is also used to treat snake bites. Heated in a little boiling water, it is patted on the head as a treatment for headache (Iwu, 2000). The bark is also used in the treatment of impotence, infertility (Iwu, 2000).

A decoction of the pounded roots is used in the treatment of intestinal problems and syphilis (Barwick, 2004). A macerate of the roots is taken by mouth as a vermifuge to rid the body of roundworm and is also used to treat hernia. Root scrapings, combined with chilli, are put into a carious tooth (Burkil, 2004).

The leaf extract is employed in the treatment of coughs, diarrhoea and dysentery, whilst it is also given to children for treating epilepsy and convulsions (Burkil, 2004). The leaf-ash, mixed with salt, is taken as remedy for heart pain. Traditionally, the leaves are cooked in palm oil soup and taken by pregnant women in to ease delivery and, after parturition, to promote a rich milk supply. Stains from the hands and fabics are believed to be removed by *Newbouldia laevis* (Burkil, 2004). It is also credited with aphrodisiac properties. During festivities and coronation ceremonies in some African communities, the leaves are used to confer traditional titles on people, and they are also used as part of masquerade costumes there.

Phytochemical analyses on the root, root bark and stem of this plant revealed the presence of alkaloids, quinoid and phenylpropanoid amongst others (Gafner *et al*., 1997; Akerele *et al*., 1998; Germann *et al*., 2006). Phytochemical composition of the flower and leaves extract revealed the presence of cardiac and steroidal glycosides, flavonoids; tannins while other phytochemicals such as alkaloids, saponins were not detected (Usman and Osuji, 2007). Based on its documented anti-oxidative propensity, NBL is assessed in the current investigation to determine its protective as well as restorative ability on wistar rat testis and liver histomorphology in cadmium-induced testicular toxicity and hepatotoxicity

## MATERIALS AND METHODS

**Animals:** All experimental procedures were conducted in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals as stated in the "Guide to the care and use of Laboratory Animals Resources". A total of thirty adult male wistar rats weighing between 150 – 250g, were used for this study. Wistar rats of the same specie *Rattus norvegicus* were procured from the animal holdings of Afe Babalola University, Ado-Ekiti, Ekiti State. The animals were kept under well-ventilated conditions, at room temperature and allowed to acclimatize for 2 weeks. During the experimental period, animals were fed Growers Mash obtained from the University Farm and allowed water ad libitum. At the end of two weeks, the rats were weighed and were randomly assigned to five different groups A as the control group B, C, D and E as the experimental groups.

**Collection and Preparation of aqueous extract from the leaves of *Newbouldia laevis*:** Fresh leaves of NBL for this study were collected from the compound of the Ewi of Ado-Ekiti, Ekiti in the month of March, 2015. The plant was identified and authenticated by Mr. G. A. Ademiriyo of the Department of Botany, Obafemi Awolowo University, Ile Ife, Osun Nigeria. A voucher specimen was deposited and a Herbarium number 17433 obtained. The leaves were harvested and properly washed in tap water and then rinsed in distilled water to remove dirt and possible mycotoxins. The leaves were air dried for five days under shade and then pulverized into fine powder using Miller III (model MS-223, China). A quantity of 220g of the powder was extracted in 1600ml of distilled water and left to stand for 48 hours at room temperature. The extract was filtered through cheese cloth and the resulting filtrate was concentrated on steam bath at 55ºC until a residue which weighed 18.2g was obtained. The percentage yielded was calculated and equivalent amount of the residue was separately

reconstituted in 141ml of normal saline to give the required doses of 200mg/kg body weight respectively.

**Induction of Toxicity:** Induction of toxicity was achieved by the oral administration of cadmium at 1.2kg/kg body weight. The induction was done orally using syringe and canula.

**Treatment:** The aqueous extract of leaves of *Newbouldia laevis* was administered orally at the dose of 200mg/kg.

**Experimental Design:** Administration was done orally using canula. The administration was regular for 30 days as follows: Group A - each rat in this group received 0.2ml per day of normal saline orally; Group B - each animal here received 1.2mg/kg body weight of cadmium chloride per day (Fabricia *et al.,* 2010); Group C- each rat in this group received 200mg/kg body weight of *Newbouldia laevis* per day (Anaduaka *et al.,* 2014); Group D - Each rat in this group received 1.2mg/kg body weight of cadmium chloride (Fabricia *et al.,* 2010) and 200mg/kg body weight of *Newbouldia laevis* (Anaduaka *et al.,* 2014) simultaneously; Group E – Animals here received 1.2mg/kg body weight of Cadmium Chloride for 15 days duration and 200mg/kg body weight of *Newbouldia laevis* for the remaining 15 days.

**Sacrification:** At the end of the experiment, the rats were sacrificed. This was done by cervical dislocation. The liver was collected and fixed in 10% formal saline. The testes were collected and fixed in Bouin's fluid.

## RESULTS

At the end of the histological analysis, marked morphological alterations were observed in cadmium-treated rats; and varying degrees of regenerations and cellular restorations were noticeable in animals treated with *Newbouldia laevis* after toxicity induction with cadmium, as presented below.

**Testicular Histology:** The testicular histology of the Control rats showed well defined testicular architecture, the lumen of the seminiferous tubule revealed numerous spermatozoa, the germinal epithelium lining the tubule and cells at different stages of spermatogenesis were defined (Plate 1). Animals treated with cadmium showed fewer cells in the lumen of the seminiferous tubule, with fewer germinal cells showing apoptosis. NBL treated rats testicular histology shows defined lumen, composed of spermatozoa (Plate 3). Tubules are composed of germ cells at different levels of maturation, sertoli cells are well defined. The blood vessel

(BV) within interstitium was free of inflammatory cells. Co-administered rats showed fewer spermatids in the lumen of the seminiferous tubules with fewer germinal cells undergoing spermatogenesis, the interstitium revealed sign of inflammation with numerous swollen blood vessels (Plate 4). Animals treated with cadmium earlier and later with NBL showed similar morphology with the co-administered animals (Plate 5).

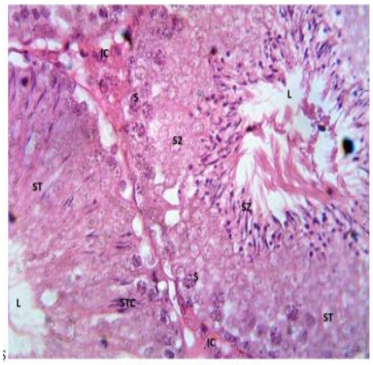


PLATE1:CONTROL TESTIS

***Micrograph (X400) showing seminiferous tubules (ST) with defined lumen (L), spermatozoa (SZ). Tubules are composed of germ cells (S2) at different levels of maturation, the Sertoli cells (STC) and interstitial cells (IC) appear essentially normal and unremarkable.***

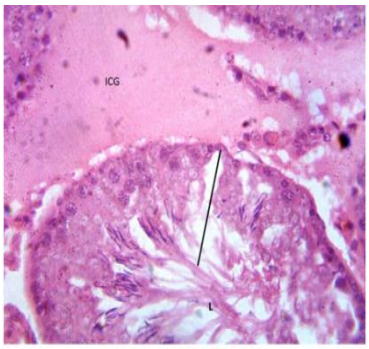


PLATE2: CADMIUM TESTIS

***Micrograph (x400) showing seminiferous tubules with decreased spermatogenic cells (line) within the lumen (L), also seen is marked congestion of the Interstitium (ICG).***

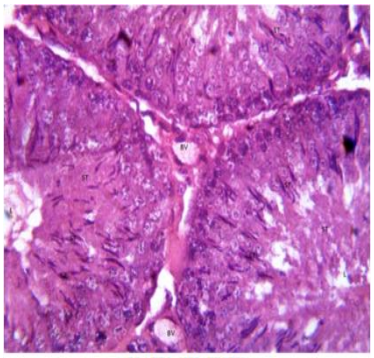

PLATE3: NBL TESTIS

***Micrograph (x400) revealed seminiferous tubules (ST) with defined lumen (L), composed spermatozoa (SZ). Tubules are composed of germ cells at different levels of maturation; the Sertoli cells (ST) are well defined. The blood vessel (BV) within Interstitium is free of collection and inflammatory cells.***

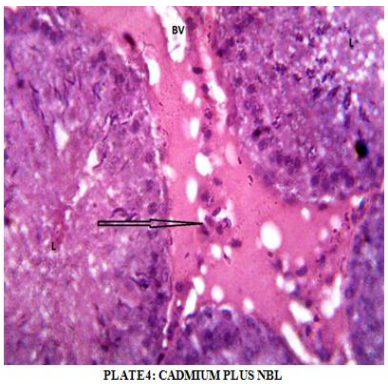


PLATE 4: CADMIUM PLUS NBL

***Micrograph (x400) showed scanty germ cells within the lumen (L), interstitial edema and proliferation of interstitial cells (arrow).***

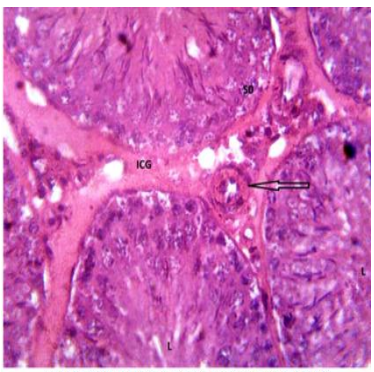


PLATE5: CADMIUM PRE-TREATED AND NBL POST-TREATED

***Micrograph (x400) shows seminiferous tubules (ST) with defined lumen (L), Interstitium congestion (ICG), thickened blood vessel (arrow) and mild proliferation of mononuclear within the Interstitium***

**Liver Histology:** Microscopic examination of the liver sections of the control rat showed normal morphological structure of the central vein and Kupffer cells, respectively as shown in Plate 6. On the other hand, microscopic investigation of the liver section of cadmium induced toxicity demonstrated hepatic tissue with marked proliferation of Kupffers cells (arrow), fat deposit (FD) and hepatocytes showing degenerative changes. Treatment with the leaves extract of NBL in the dose of 200mg/kg body weight showed partial/mild restoration of normal histological structure of the liver with few disturbances in the liver cells (Plate 10).

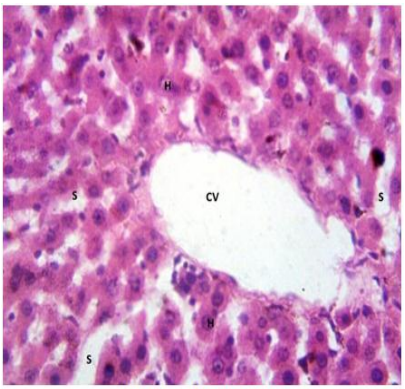


PLATE 6: CONTROL LIVER

***Micrograph (x100) shows a hepatic tissue with central vein (CV) located at the center of the lobule; hepatocytes (H) disposed in sheet, and separated by the sinusoids (S). The Interstitium it appears essentially unremarkable***

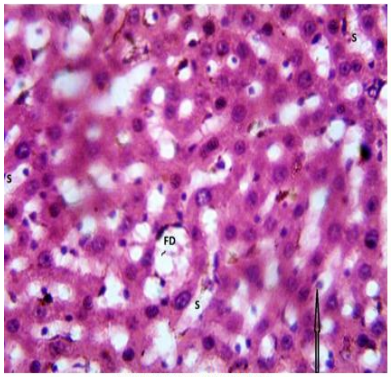


PLATE 7: CADMIUM LIVER

***Micrograph (x100) shows hepatic tissue with marked proliferation of Kupffers cells (arrow). Hepatocytes show degenerative changes.***

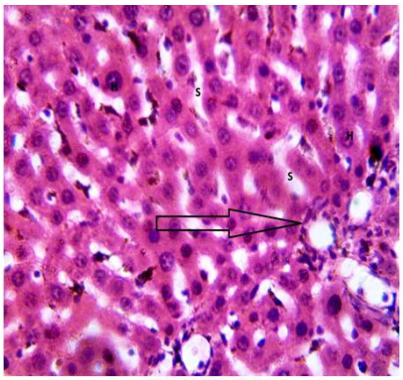


PLATE 8: NBL LIVER

***Micrograph (x100) shows mild infiltration of the portal region with polymorphs (arrow), the vascular channels are free of collection and inflammatory cells.***

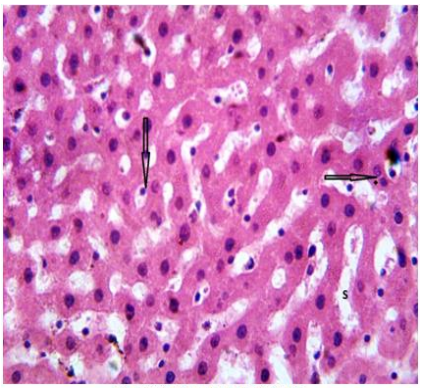


PLATE 9: CADMIUM PLUS NBL

***Micrographs (x100) shows activation of the Kupffers cells, (vertical arrow), binucleated hepatocytes (horizontal arrow). The sinusoids are free of collections. Hepatocytes shows regenerative changes***

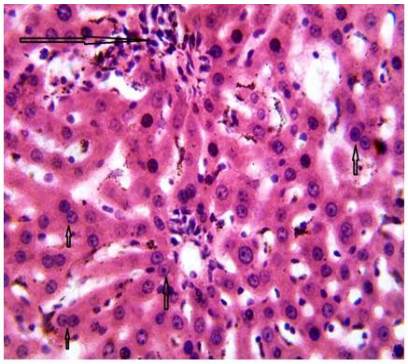

PLATE 10: CADMIUM PRE-TREATED AND NBL POST-TREATED

***Micrographs (x100) shows activation of the kupffers cells, (vertical arrow), binucleated hepatocytes (vertical arrows). There is mild infiltrate of inflammatory cells (horizontal arrow).***

## DISCUSSION

Environmental toxicity has been shown to be part of pathogenesis of some illness in the world now (ATSDR, 2008). Fertility problems have been shown to correlate greatly with the present of environmental toxins present in the body. Cadmium, a heavy metal present in the atmosphere as a result of industrial pollution has been shown to penetrate the biological system through inhalation (Singh *et al*., 2007). Several studies have shown that cadmium affect cellular metabolism by generation of Reactive Oxygen Species (ROS) or inhibition of cellular respiratory enzymes (Liu *et al*., 2001), thereby affecting the cellular function and with more exposure leads to cell death. From the present study, exposing rats to 1.2mg/kg body weight of cadmium orally for 4 weeks affected the testicular morphology by fewer spermatids in the lumen of the seminiferous tubules and altered germinal cells morphology. This shows that cadmium may affect the spermatogenesis process either by affecting the

survival of the germinal cells or affecting the hormones responsible for spermatogenesis (El-Shahat *et al*., 2009). Although reproductive hormonal assay was not done in this work, there is a possibility that cadmium may affect the hypothalamus which secretes gonadotropin releasing hormone as it has been reported that cadmium induces neurotoxicity (Lafuente *et al*.,2001) leading to hormonal imbalance.

Inflammation is a physiological process for cellular survival which becomes toxic in some conditions and leads to cellular damage (Usman and Osuji, 2007). Most toxic substances may also induce inflammatory processes. Alleviating the effect of toxic substance in the biological system makes some researchers to do more work on anti-oxidants. NBL has been shown to have many anti-oxidants properties (Usman and Osuji, 2007). The phytochemical analysis has shown NBL to have flavanoids, saponins, alkaloids, glycosides, *etc.* (Usman and Osuji, 2007). I tried to check if NBL can inhibit cadmium induced testicular toxicity. The histological results revealed that NBL treated rat has normal testicular morphology as compared with the control. Co-administered and later treated group still showed testicular damage. From the sections above, it is revealed that NBL is unable to reverse the testicular damage caused by cadmium. Most researchers reported that NBL is a potent anti-oxidant; it is shown here that NBL is unable to reverse the testicular damage done by cadmium. This may be due to the fact that testicular damage done by cadmium may not be through the ROS formation alone as reported by some researchers. Since animals given NBL alone did not show testicular disruption, it then suggests that NBL is non-toxic. However, it will be worthy of note to mention that the reckless consumption of NBL with other substances mixed together for the purpose of treating infertility should be critically watched as this study has revealed further testicular damage in the rats treated with both cadmium and NBL. The liver is the primary target organ following acute systemic cadmium exposure. The uptake of Cadmium into the liver is critical for the development of overall toxicity induced by the heavy metal. Approximately half of cadmium absorbed systemically is rapidly accumulated in the liver (DelRaso *et al*., 2003). In the present study, administration of cadmium resulted in severe hepatocyte necrosis, fatty changes, degeneration signs and inflammatory cell infiltrations. These results were similar to the acute and chronic effects of cadmium documented by previous studies (Gong *et al.*, 2008; Ersan *et al.*, 2008; Renugadevi and Prabu, 2010). The histopathological changes of the liver treated with cadmium might be due to the formation of highly reactive radicals and subsequent lipid peroxidation induced by cadmium. The accumulated hydroperoxidase can cause cytotoxicity, which is associated with

the peroxidation of membrane phospholipids by lipid hydroperoxidase, the basis of hepatocellular damage (Renugadevi and Prabu, 2010).The Co-administered, Pre-treated and Post-treated groups showed binuclated hepatocytes, mild infiltration of inflammatory cells which further explain the NBL ameliorated histopathological damage induced by cadmium. The NBL reduces the toxicity and absorption of cadmium.

## SUMMARY/CONCLUSION

In conclusion, the result of this investigation revealed that the exposure of experimental rats to cadmium has deleterious effects on its major organs (testes and liver). The administration of aqueous leaf extracts of *Newbouldia laevis* may be used to manage hepatotoxicity and testicular toxicity induced by cadmium; however, further work should be done to determine the actual pattern through which cadmium induces its toxicity on the testes. The bodies of evidences therefore, suggest that NBL is non-toxic but the reckless consumption with other mixtures for the purpose of treating infertility or other medical condition should be checked as it can induce testicular damage as demonstrated in the present study. I equally recommend, for a more appreciable result, biochemical and immunohistochemical analyses be done in subsequent studies.

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